Shah et al., Supplemental Methods

Animal Experiments

Mice were housed in a pathogen-free facility at the University of Iowa on a 12-h light/ dark cycle. AAV8 virus was suspended in phosphate-buffered saline and injected intraperitoneally. TM or vehicle (DMSO) was diluted in phosphate-buffered saline and was injected intraperitoneally. Tissue was either flash frozen for isolation of protein or RNA, or fixed in formalin (for histological analysis) or glutaraldehyde (for TEM). Immunohistochemistry (IHC) of samples fixed in 4% neutral buffer formalin-fixed liver tissue was performed by the Comparative Pathology Laboratory (CPL), University of Iowa. For TEM, a small piece of liver tissue was finely minced and fixed using 2.5% glutaraldehyde and stored at 4°C. Samples were further processed for TEM images by the Central Microscopy Research Facility, University of Iowa. Blood was collected via the retro-orbital route. RNA was purified using Trizol according to the manufacturer's instructions (ThermoFisher), and protein lysates were collected by homogenization in RIPA buffer, followed by sedimentation of insoluble material, removal of the fat cap, addition of 5x loading buffer, and boiling. For real-time RT-PCR, C_t values were normalized against the average of two housekeeping genes (*Btf3* and *Ppia*) to mitigate the possibility of changes in a housekeeping gene confounding the expression quantification.

RNA-seq

RNA seq data quality was analyzed using FastQC (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/, v.0.11.8). The FASTQ RNA-seq files were aligned to Ensembles Transcriptomes v.94. for *Mus musculus* (https://useast.ensembl.org/info/data/ftp/index.html). A pseudo alignment program *Kallisto* [v_0.45.0; (50)] was used to align the RNA seq FastQC data files to determine the transcript abundances. Downstream differential gene expression analysis was performed by *DESeq2* [v_1.34.0] (51) using the *R Studio* statistical package (v_4.1.2). All the dependencies required for the analysis of RNA seq data were gathered from the Bioconductor site (https://www.bioconductor.org). Gene set enrichment was performed by using GSEA software (http://www.gsea-msigdb.org/gsea/index.jsp; GSEA_4.0.3) following the pre-ranked method. Normalized differentially expressed genes from DESeq2 analysis were ranked based on their $\log_2$FC. The hallmark gene v7.1 for pre-ranked method and gene symbol remapping 7.1. and a default setting was used for analysis.

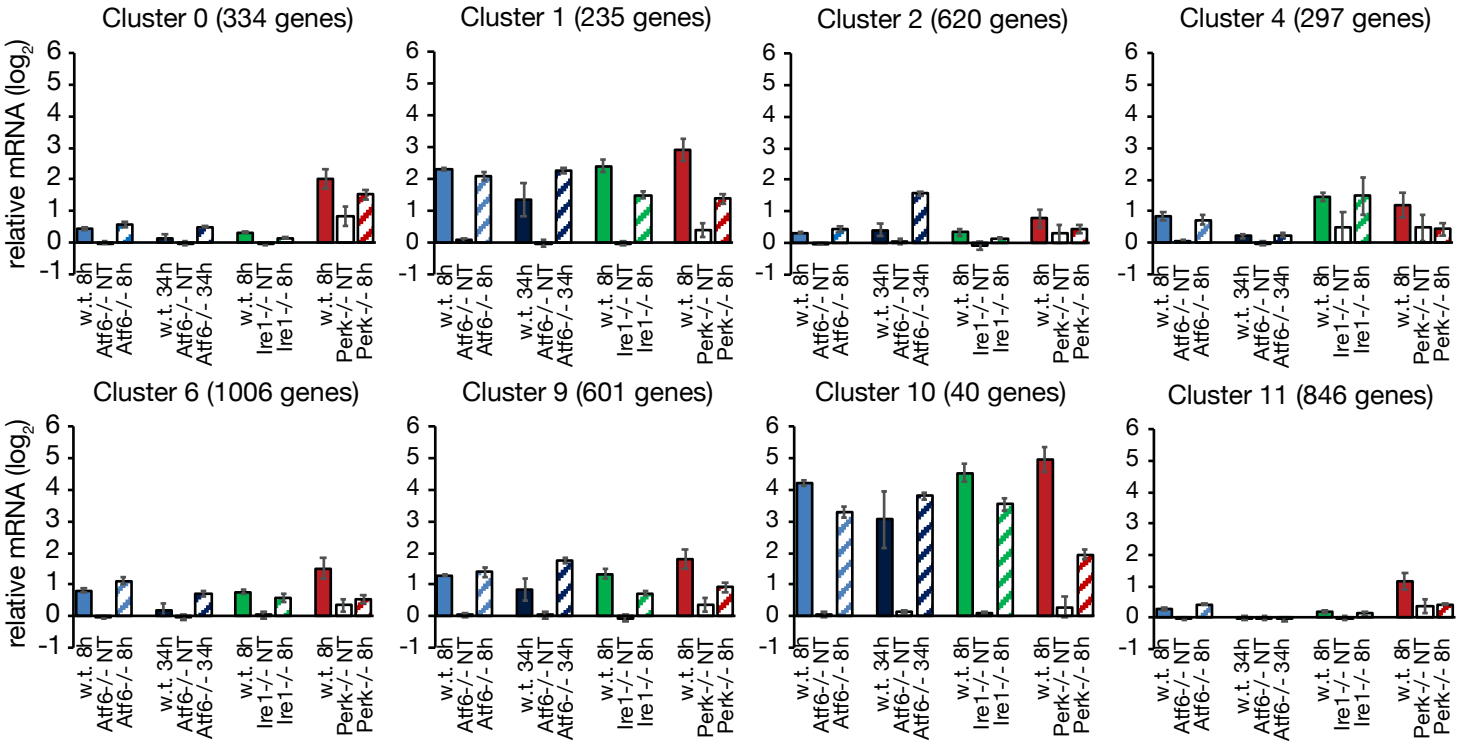

Figure S1. Centroids for upregulated genes from k-means clustering

Genes that were upregulated by ER stress from the microarray datasets were partitioned into 8 of the 12 clusters. The centroids for these genes and the number of genes in each cluster are shown here. Because some genes in the microarray are represented by more than one probeset, and because in some instances these different probesets do not behave identically, a small number of genes are present in more than one cluster.

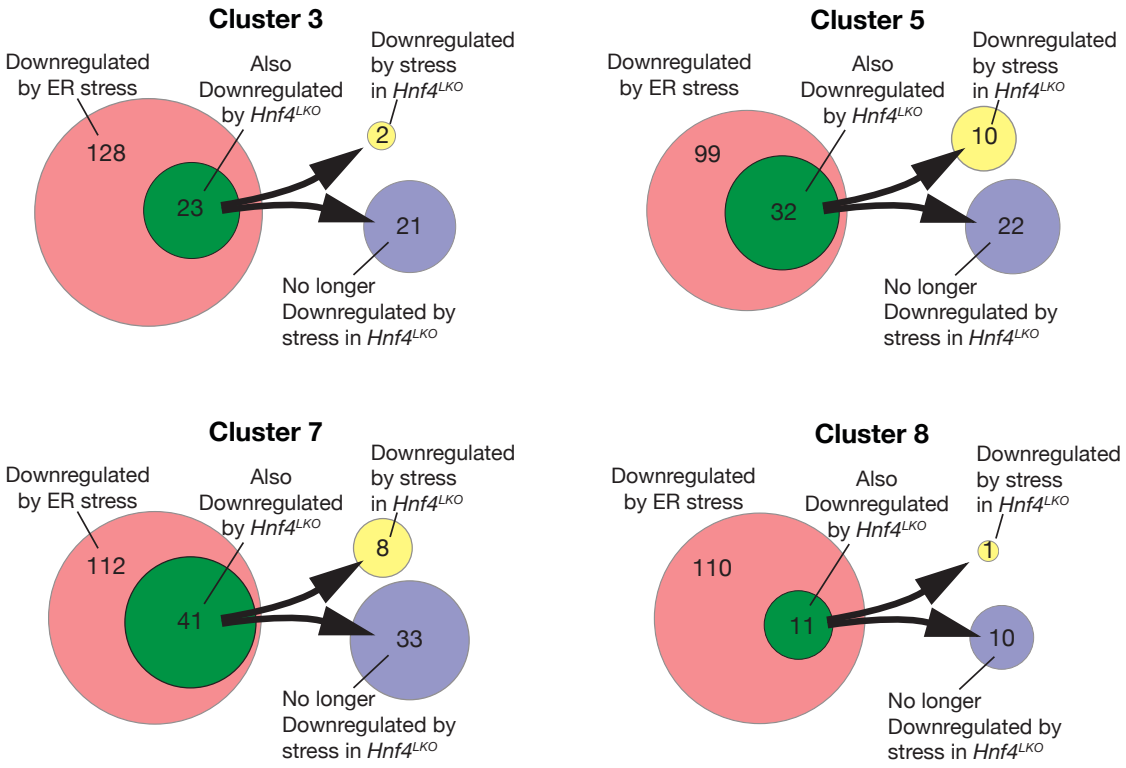

Figure S2. Stress regulation of HNF4α-dependent genes from clusters 3, 5, 7, and 8

Red circles represent genes from each cluster that were confirmed to be significantly downregulated by TM treatment in wild-type animals from RNA-seq dataset. Green circles represent the genes within that group that were also significantly downregulated by loss of HNF4α. The genes of that group were subdivided into those that were no longer downregualted by TM in *Hnf4α*^{LKO} animals (blue circles) and those that were further downregulated in the same conditions (yellow circles).

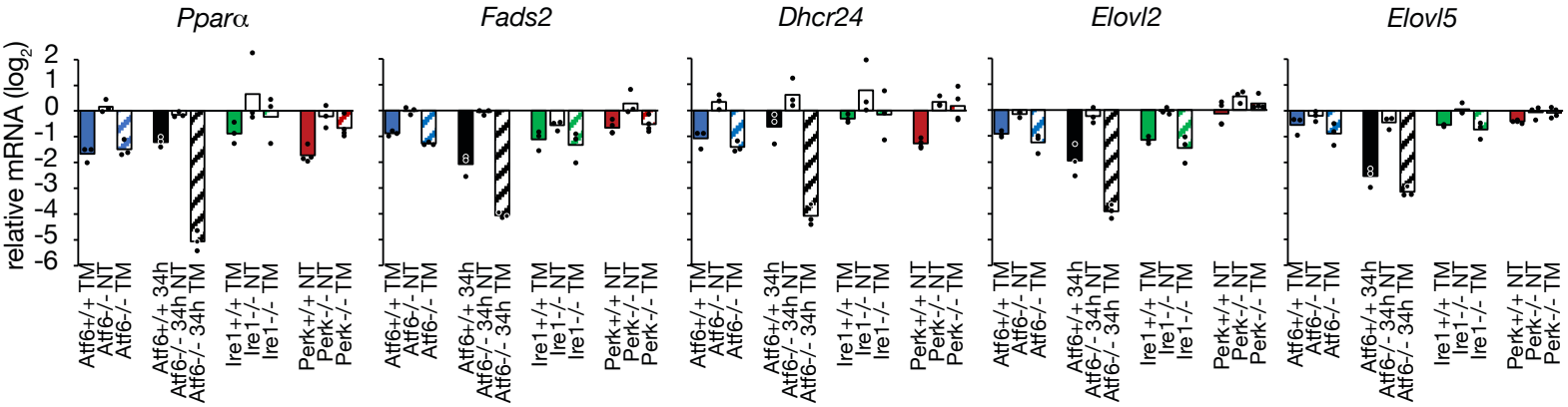

Figure S3. Expression of selected cluster 7 genes in microarray data

The behavior of the 5 cluster 7 genes shown in Figure 3B in the microarray datasets from Figure 1 is shown here.

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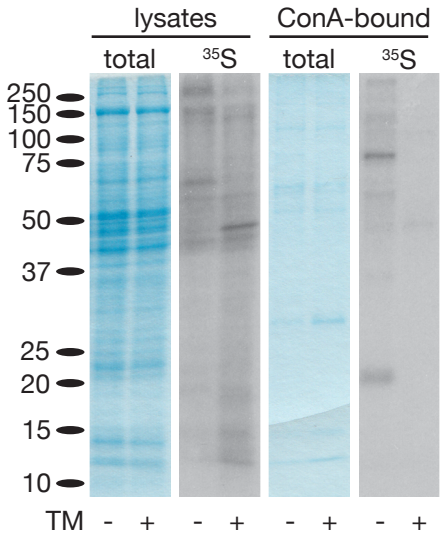

Figure S4. Selectivity of ConA for nascent N-linked glycoproteins

Primary hepatocytes treated with vehicle or 5 μg/ml TM were labeled with ^{35}S-Met/Cys for 30 minutes. An aliquot of each lysate was set aside and the remainder was bound to ConA-sepharose and then eluted with α-D-methyl-mannopyranoside. Samples were visualized by Coomassie stain or autoradiography. TM prevented the ConA binding of almost all nascent proteins but had little or no effect on total binding of N-linked glycoproteins (since the drug has no effect on the glycosylation status of pre-existing glycoproteins), demonstrating selectivity of the ConA resin for nascent glycoproteins.